

DIEL PERIODICITY OF MALE SEX PHEROMONE RESPONSE AND FEMALE ATTRACTIVENESS IN THE GYPSY MOTH (LEPIDOPTERA: LYMANTRIIDAE)¹

R. T. CARDÉ

Department of Entomology, New York State Agricultural Experiment Station, Geneva

C. C. DOANE

Department of Entomology, Connecticut Agricultural Experiment Station, New Haven

W. L. ROELOFS

Department of Entomology, New York State Agricultural Experiment Station, Geneva

Abstract

Can. Ent. 106: 479–484 (1974)

In field studies gypsy moth males were attracted to synthetic *cis*-7,8-epoxy-2-methyloctadecane (disparlure), the female sex pheromone, and virgin females from 0900 to 2000 (Eastern Standard Time). The greatest numbers of males were lured to the synthetic attractant or the calling female from 1100 to 1500. These periods of male response are longer than reported in previous (1896 and 1932) New England investigations and suggest the possible recent evolution of a new diel rhythm of male sex pheromone response.

Introduction

Although there have been many investigations of the pheromonal biology of the gypsy moth, *Porthetria dispar* (L.), the rhythms of male attraction to the female sex pheromone and the calling female have not been studied in detail. This paper describes the periodicity of male attraction to *cis*-7,8-epoxy-2-methyloctadecane (disparlure), the sex pheromone of the female gypsy moth (Bierl *et al.* 1970), and the periods during which the female attracts males.

Materials and Methods

The study areas in 1971 in Haddam (southern Connecticut) and in 1973 in Somers (northern Connecticut) were both deciduous woodland consisting predominantly of red and white oaks (*Quercus rubra* L. and *Q. alba* L.). Larval defoliation of approximately 30 to 40% resulted in moderate adult populations that produced many males but comparatively few females. The synthetic pheromone used in 1971 and 1973 was 97.3 and 94.0% pure, respectively, by gas chromatography. Test chemicals were placed either in polyethylene caps (OS-6 Natural Polyethylene Closures, Scientific Products) or on rubber septa (5×9 mm rubber stoppers, sleeve-type, Arthur H. Thomas, Co.). These dispensers were positioned in the bottom center of white XC-26 Sectar® traps (10×10 cm diamond-shaped opening and 23 cm in length with an internal sticky surface, 3M Co.).

In 1971, virgin females were obtained from field-collected pupae maintained until emergence in the laboratory at 23°C, 65% R.H., and with a light:dark cycle corresponding to natural sunrise and sunset. On each day freshly eclosed (0–24 h old) females were brought to the test area and a single female was enclosed in a cylindrical cage 7 cm high and 4 cm in diameter formed from copper screening. Cages containing the females were placed in the traps at 0900 (all times reported in this study are Eastern Standard Time). In 1973 the procedure was similar except that pupae and emerged females were held outside the test area under natural photoperiod and temperature until use.

¹Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 2025. Supported in part by the Rockefeller Foundation.

The 1971 tests were conducted from 0900 15 July to 0900 17 July. During July the mean high was $27.6 \pm 1.9^{\circ}\text{C}$ (S.D.), while the mean low was 14.9 ± 2.3 S.D. On the 1971 study days the maxima-minima were 26.7 to 13.3 and 28.4 to 15.0. In 1973 observations were conducted from 0900 23 July to 0900 24 July when the temperature ranged from 27.2 to 11.7. For this area the July mean high was 27.7 ± 3.3 S.D. while the mean low was 15.6 ± 3.7 S.D. On the experimental days there was little or no cloud cover.

In 1971, treatments were replicated 5 times in a complete randomized block design. Within replicates, treatments were 8 m apart and replicate blocks were separated by 100 m or more. In 1973, treatments were replicated 8 times and replicate blocks were separated by 20 m or more. Traps were hung on the branches of small trees at a height of 1.5 m and moths captured were counted hourly during periods of activity and at 2-h intervals at other times. Data (Fig. 1) from 1971 were plotted to the nearest 2-h interval, but observations of the initiation and termination of attraction reflect actual timing. Although traps had a capacity of ca. 50 moths, efficiency of capture was lowered somewhat after traps held more than 15–20 moths. High trapping efficiency (i.e., males observed entering the traps were captured) was maintained by removing captured males with forceps and by replacing traps that had appreciable quantities of wing scales on the sticky surface. Differences in treatment means were determined by an analysis of variance.

Results

1. *Diel Periodicity of Male Attraction*

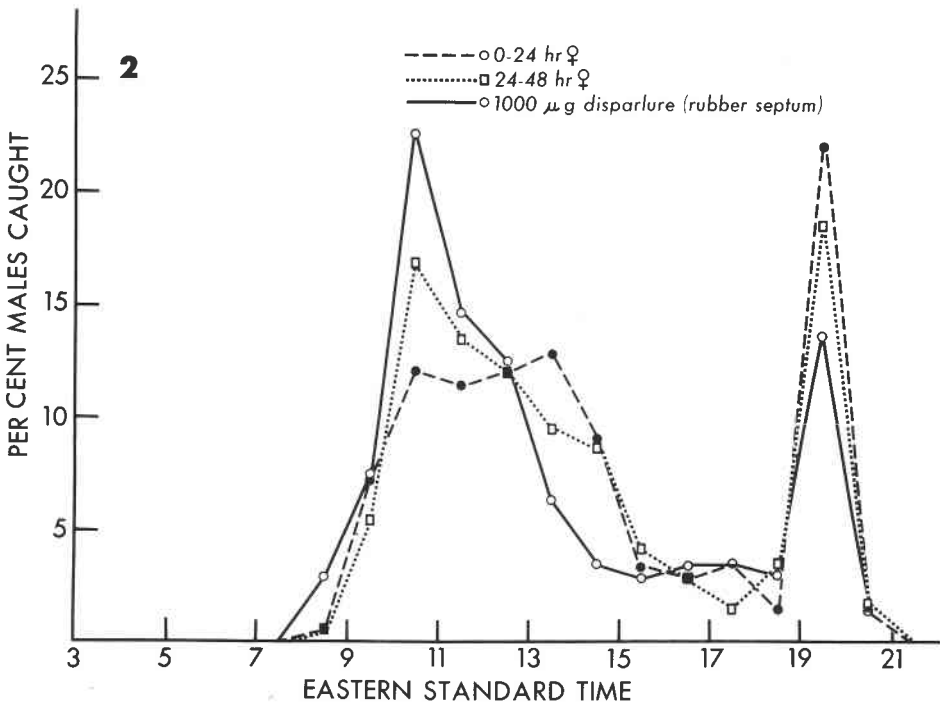
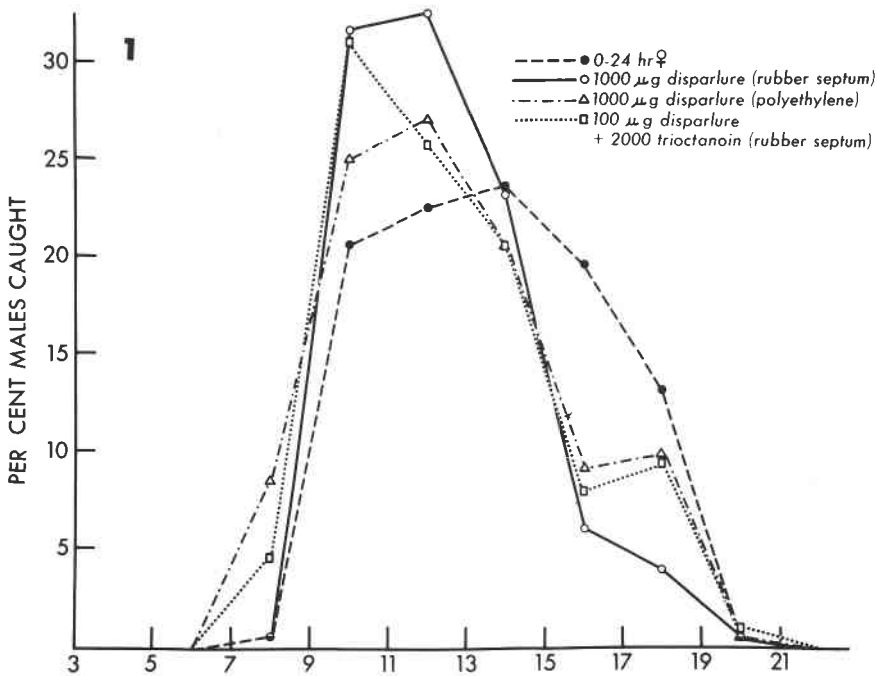
Field responses in 1971 (Fig. 1) and 1973 (Fig. 2) indicate that in some males attraction response to synthetic disparlure and live females is initiated between 0800 and 0900. The greatest numbers of males were lured both to the disparlure and the females from approximately 1100 to 1500. In 1971 and 1973 male attraction response continued until 2000, when males apparently were no longer responsive. Comparison of the intervals from 1500 to 2000 in 1971 and 1973 reveals two distinct patterns. In 1971 male response after 1600 tapered off gradually, whereas in 1973 a second response maximum between 1900 and 2000 is evident. During this particular interval, the eight 0–24 h and the eight 24–48 h females lured means of 5.8 (± 2.8 S.E.) and 5.8 (± 1.5 S.E.) males, respectively, with all females luring at least one male. The synthetic pheromone traps captured a mean of 2.5 (± 0.6 S.E.) males with only one of eight traps failing to lure a male.

2. *Relative Attractiveness of Synthetic Pheromone and Females*

In the 1971 test, comparison (Table I) of the various formulations of disparlure with the calling female reveals that the latter was more attractive than any of the pheromone dispensers employed. When the relative attractiveness of the virgin female was contrasted to the synthetic pheromone at various times within the male response interval (Table I and Fig. 1), it is evident that the comparative attractiveness of the female increases toward the end of the response interval. The same trend is present in the 1973 test (Fig. 2).

Discussion

Previous investigations of gypsy moth behavior in New England have indicated that attraction and mating occurs in the daylight hours. Forbush and Fernald (1896) reported that male flight and attraction to females was initiated at 0900, and that the period of greatest male activity occurred between 1000 and 1500 to 1600, after which there was little voluntary male flight. Collins and Potts (1932)



FIGS. 1-2. 1, periodicity of male attraction to various pheromone dispensers and individual 0-24 h live females from 0900 15 July to 0900 17 July, 1971. Sunrise was at 0430 and sunset at 1928. Treatments replicated 5 times and treatment means given in Table I. 2, periodicity of male attraction to pheromone and individual live females from 0900 23 July to 0900 24 July, 1973. Total males attracted to 0-24 h females, 201; to 24-48 h females, 256; and to pheromone, 137. Treatments replicated 8 times; unbaited traps caught no males.

Table I. Comparative attractiveness of *cis*-7,8-epoxy-2-methyloctadecane (disparlure) and virgin females, 1971. Differences at the 5% level among means in each column are indicated by different letters

Treatment	Dispenser	Mean males/trap	Mean males/trap \pm S.E.		
		0900 15 July– 0900 17 July	0900–1200 16 July	1200–1500 16 July	1500–1900 16 July
Single female		163.2 _a	35.0 _a \pm 4.0	40.4 _a \pm 7.8	28.8 _a \pm 8.4
1000 μ g disparlure	Polyethylene	114.6 _b	21.6 _b \pm 3.1	20.4 _b \pm 3.5	7.0 _b \pm 3.2
1000 μ g disparlure	Rubber	89.0 _b	22.8 _b \pm 1.9	14.8 _b \pm 5.3	4.4 _b \pm 2.1
96 μ g disparlure + 2000 trioctanoin	Rubber	93.8 _b	22.8 _b \pm 4.0	13.8 _b \pm 3.4	9.8 _b \pm 5.4

reported that males were lured to females and female abdominal tip extracts from 0900 to as late as 1800. Our findings suggest that the intervals of male response and possibly female attractiveness may be longer now (0900 to 2000) than reported in the two previous New England investigations.

Investigations in Europe have yielded rather disparate findings as to the periodicity of male attraction and female attractiveness in the gypsy moth. Although two reviews (Jacobson 1965, 1972) indicate that mating in this species usually occurs during the evening and night, the supporting European literature is somewhat ambiguous. Jacentkovský (1932, 1934) observed that attraction to the female occurred principally during the daylight hours, with the peak response around midday. Prüffer (1923) indicated that the male's active search for the female takes place during the day, although under some experimental conditions mating could occur in the early evening. Görnitz (1949) reported that in cage tests pheromone stimulation may have taken place at night, and that in the field male attraction occurs in late afternoon and evening.

The close congruence of the temporal patterns of attraction response of males to virgin females and synthetic attractant suggest that a key factor in the periodicity of male attraction to females is the rhythm of male response rather than rhythm of female calling and pheromone secretion. Of particular interest is that in the 1973 observations all 16 females and 7 of 8 disparlure-baited traps attracted males from 1900 to 2000. As the pheromone-baited traps were emitting pheromone continuously, initiation and cessation of male attraction to females was not limited by the female's temporal pattern of pheromone emission. These data also suggest that a gypsy moth control program utilizing pheromone-baited traps would not have much daily temporal advantage over native females.

The temporal differences in the comparative attractiveness of females and synthetic may be the result of differential emission of pheromone by the female. Richerson and Cameron (in press) have found in laboratory analyses of field-collected females that maximal release of disparlure occurs between about 1100 and 1500, although some pheromone is emitted at all times.

The role of temperature in influencing the timing of male attraction was not investigated directly. It is known in other Lepidoptera that ambient temperature can modify the rhythms of female calling, or male response, or both, as has been shown in an arctiid (Cardé 1971; Cardé and Roelofs 1973), numerous tortricids (Comeau 1971; Sanders and Lucuik 1972), and a noctuid (Sower *et al.* 1971). In

these cases mating rhythms on cool summer or spring days occur earlier than those at warm summer temperatures, such that the period of activity is shifted forward to the warmer afternoon or early evening. In the gypsy moth the effect of cool days would likely be the shortening of the response interval, as suggested by Collins and Potts (1932). These authors indicate that temperatures below 20.6 eliminate voluntary male flight, as does rain or wet foliage.

In Connecticut, adults are present from early July to mid-August, dependent on geographical location, weather, and the larval population level which influences the rate of development (Leonard 1970). In the 1971 and 1973 study areas, adults occurred from approximately 5 July to the end of July. The temperature maxima-minima for the three observation days were typical of local climatic conditions during the entire flight periods, but it is possible that subtle fluctuations in the temperature regimes account for the differences in the 1971 and 1973 data.

Although the numbers of males captured after 1600 are significant, it is not certain how these numbers relate to the actual proportion of males that are responsive at this time. Time-dependent variation in the threshold of pheromone response or in flight behavior within the diel response period might affect the numbers attracted at any given time. Also, dense adult female populations may result in the emission of enough pheromone into the environment to modify male behavioral patterns.

In dense populations, we have observed that females generally are mated before 1600, and consequently a lengthened rhythm of attraction response may not be of great adaptive significance unless females tend to emerge late in the day. But a more pertinent consideration may be the mating success of males and females in sparse populations, which it is thought serve as foci for incipient outbreaks (Leonard 1970). In such discontinuous distributions, a lengthened searching interval could effect an increased mating success as females would not tend to be mated soon after emergence as they are in dense populations.

Another reason for a distinct North American rhythm in part may be due to the founder effect (Mayr 1963). The gypsy moth was introduced into Massachusetts in 1868 or 1869. The founder population gene pool was likely quite small (Forbush and Fernald 1896) and perhaps this propagule was unrepresentative of the gene pool of the European parent population in terms of behavioral characteristics. A salient factor contributory to the apparent rhythm lengthening may have been reduced pheromonal communication interference: in New England no other Lepidoptera are known to employ the same attractant chemical. In Europe and Asia the gypsy moth is widely sympatric with *Lymantria monacha* L., the nun moth, and both of these closely-related lymantriids apparently employ the same attractant chemical since both Schönherr (1972) and Schneider *et al.* (1974) have found the nun moth attracted to disparlure. The nun moth has been reported to mate from 1800 to 0100 (Ambros 1938, 1940) and from 1900 to 2100 (Nolte 1940), suggesting some temporal isolation with the gypsy moth. Mating periodicities of these species in Europe need further investigation (particularly as previous observations were not designed to delineate periodicity). Notwithstanding, in Europe it does appear that differing mating rhythms in these species are a potential barrier to cross-communication. The absence in New England of selection against gypsy moths mating in the early evening may have been a factor in the apparent extension by about 4 h of the diel periodicity of male response.

Acknowledgments

We thank Dr. J. Tette (Zoecon Corporation) for supplying the disparlure used in 1971 and Dr. A. Hill for synthesis of the disparlure used in 1973. Drs. E. A. Cameron and J. V. Richerson (Pennsylvania State University) kindly allowed use of unpublished data and commented on this paper.

References

- Ambros, W. 1938. Einige spezielle Beobachtungen und Untersuchungen während der Nonnenkontrolle in Jahre 1937. *Z. ges. Fortwesen* **64**: 49–60.
- 1940. Einige Beobachtungen und Untersuchungen an der Nonne im Jahre 1938. *Z. ges. Fortwesen* **66**: 131–147; 166–169.
- Bierl, B.A., M. Beroza, and C. W. Collier. 1970. Potent sex attractant of the gypsy moth, *Porthetria dispar* (L.): Its isolation, identification and synthesis. *Science* **170**: 87–89.
- Cardé, R. T. 1971 (unpub.). Aspects of reproductive isolation in the *Holomelina au-antiaca* complex (Lepidoptera: Arctiidae). Ph.D. Thesis, Cornell University.
- Cardé, R. T. and W. L. Roelofs. 1973. Temperature modification of male sex pheromone response and factors affecting female calling in *Holomelina immaculata* (Lepidoptera: Arctiidae). *Can. Ent.* **105**: 1505–1512.
- Collins, C. W. and S. F. Potts. 1932. Attractants for the flying gypsy moths as an aid in locating new infestations. *U.S. Dep. Agric. Tech. Bull.* 336. 43 pp.
- Comeau, A. 1971 (unpub.). Physiology of sex pheromone attraction in Tortricidae and other Lepidoptera (Herterocera). Ph.D. Thesis, Cornell University.
- Forbush, E. H. and C. H. Fernald. 1896. The gypsy moth. Wright and Potter Printing Co., Boston. 495 pp.
- Görnitz, K. 1949. Anlockversuche mit dem weiblichen Sexualduftstoff des Schwammspinners (*Lymantria dispar*) und der Nonne (*Lymantria monacha*). *Anz. Schädlingsk.* **22**: 145–149.
- Jacentkovský, D. 1932. Nový způsob kontroly mnišky. *Les. Pr.* **11**: 149–152.
- 1934. Výskyt bekyně vekohlavé (*Liparis dispar* L.) v plesí Rafajna (Podk. Rus.). *Les. Pr.* **13**: 27–36.
- Jacobson, M. 1965. Insect sex attractants. Interscience (Wiley), New York. 154 pp.
- 1972. Insect sex pheromones. Academic Press, New York. 382 pp.
- Leonard, D. E. 1970. Intrinsic factors causing qualitative changes in populations of *Porthetria dispar* (Lepidoptera: Lymantriidae). *Can. Ent.* **102**: 239–249.
- Mayr, E. 1963. Animal species and evolution. Belknap Press, Cambridge. 797 pp.
- Nolte, H. W. 1940. Neue Erfahrungen zur Dykschen Nonnenanlockmethode. *Z. ges. Forstwesen* **66**: 197–206; 252–267.
- Prüffer, J. 1923 (1924). Observations et expériences due les phéromènes de la vie sexuelle de *Lymantria dispar* L. *Bull. int. Acad. pol. Sci. Lett.* (B) **1**: 1–9.
- Richerson, J. V. and E. A. Cameron. 1974. Differences in pheromone release and sexual behavior between laboratory reared and wild gypsy moth adults. *Envir. Ent.* (in press).
- Sanders, C. J. and G. S. Lucuik. 1972. Factors affecting calling by female eastern spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Can. Ent.* **104**: 1751–1762.
- Schneider, D., R. Lange, F. Schwarz, M. Beroza, and B. A. Bierl. 1974. Attraction of male gypsy and nun moths to disparlure and some of its chemical analogues. *Oecologia* **14**: 19–36.
- Schönherr, J. 1972. Die Wirkung von Disparlure auf die Nonne, *Lymantria monacha* L. *Z. angew. ent.* **71**: 260–263.
- Sower, L. L., H. H. Shorey, and L. K. Gaston. 1971. Sex pheromones of noctuid moths. XXV. Effects of temperature and photoperiod on circadian rhythms of sex pheromone release by females of *Trichoplusia ni*. *Ann. ent. Soc. Am.* **64**: 488–492.

(Received 18 May 1973)